IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Richard F. Selden *et al.* Art Unit : 1652
Serial No. : 09/686,497 Examiner : N. Nashed
Filed : October 11, 2000
Title : OPTIMIZED MESSENGER RNA

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY BRIEF PURSUANT TO 37 C.F.R. § 41.41

Responsive to the Examiner's Answer ("Answer") mailed August 19, 2004, Appellants reaffirm the arguments previously submitted in the Brief In Appeal ("Brief") filed June 28, 2004, and respond to the new points raised in the Answer as follows.

Rejection Under 35 U.S.C. § 103(a)

In the Answer, the rejection is maintained in view of Seed, Kim, Morgan, Bishop and Wada. Appellants reiterate that the Examiner has not established a *prima facie* case of obviousness. This position is not inconsistent with the prosecution history, as Appellants argued that the Examiner did not establish a *prima facie* case of obviousness in the Response to Office Action filed March 5, 2003. Appellants maintain that position. As discussed below, the references cited by the Examiner provide absolutely no motivation to skilled practitioners to combine the references and arrive at the claimed

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

October 19, 2004
Date of Deposit

Signature

Lisa G. Gray
Typed or Printed Name of Person Signing Certificate

invention with any reasonable expectation of success, and also teach away from the claimed invention.

Seed

The Examiner misconstrues the teachings of Seed. According to the Examiner (Answer at page 3), Seed teaches

> a synthetic mammalian gene in which at least one non-preferred or less preferred codon is replaced with a mammalian preferred codon...[and] that said mammalian gene is expressed at much higher level which is 110%, 150%, 200%, 500%, 1000% or 10,000% relative to that of the wild-type mammalian gene, see page 2, lines 10-16.

Appellants respectfully disagree. Seed provides no evidence whatsoever that a mammalian gene, in which less preferred codons are replaced with preferred codons, is expressed at the levels recited in the claims. The above-quoted passage does not come from a peer-reviewed publication but is merely text from the specification of Seed's published patent application. In the application, Seed only describes experiments that analyzed the expression levels of a viral gene (HIV-1 gp120) in which the less preferred codons were replaced with preferred codons. Thus, the teachings of Seed are not as broad as the Examiner asserts.

The Examiner further characterizes Seed as teaching a "highly optimized gene normally expressed in human cells, i.e., HIV-1 gp120 and another gene normally expressed in jellyfish" (Answer at page 6). The Examiner concludes (Answer at page 7)

> Thus, the teaching of Seed is sufficiently detailed to guide one of ordinary skill in the art construct [*sic.*] a synthetic gene in which all or the vast majority of the human non- and un-preferred codons are substituted with the human preferred codons (emphasis added).

Appellants respectfully disagree with the Examiner's conclusion regarding the optimization of any synthetic gene. Seed only teaches that an exogenous viral protein can be expressed at high levels in a human cell following optimization of the viral gene. Although HIV-1 gp120 can be expressed in human cells, it is only expressed in cells that have been infected by HIV-1. It is well known that viral infection results in cellular

changes that have profound effects on protein expression. Thus, as Appellants have pointed out (Brief at page 6), replacing non-preferred codons in a particular endogenously expressed human sequence with common human codons is a completely different concept than replacing codons in an exogenous non-human viral sequence with common human codons. Contrary to the Examiner's conclusion, Seed provides no guidance regarding the optimization of an endogenous human gene, specifically α-galactosidase, to increase expression in human cells.

The Examiner disagrees with Appellants' position and states that "[t]he prior art of record as well as the state of the art show that any gene from any source can be expressed in any cell at high level [*sic.*]" (Answer at page 8). This proposition is exceedingly broad and, contrary to the Examiner's assertion, it is not supported by the prior art of record. In the Office Action mailed November 18, 2002, the Examiner stated (at page 5)

> applicants should note that any α-galactosidase gene from any biological source can be optimized for express [*sic.*] in almost any host cell including human, *E. coli*, yeast and insect among others because the common codons for many organisms are known, see Wada et al.

Appellants respectfully disagree with the Examiner. Wada simply provides a table summarizing the codon usage for different organisms. Nothing in Wada teaches or suggests the proposition that, based on knowledge of the codon usage of different organisms, any protein from any biological source can be optimized to be expressed in any host cell. While it may be technically true that, based on Wada, one could have synthesized a gene in which any or all of the codons were replaced with the common codons from a particular species, there is no expectation of success of increased protein expression of a human α-galactosidase (that has been optimized to the very high levels required by the claims) in human cells. Further, the Examiner has not provided any objective evidence to demonstrate that the state of the art at the time of filing supports the Examiner's broad proposition.

In the Appeal Brief, Appellants pointed out that native human sequences perform well in the context in which they evolved. The Examiner states (Answer at page 8) that

> Applicants would like us to believe "human sequence has been already optimized by nature", see page 6 of the brief, lines 8 and 9 from the bottom of the page. This is simply not true. There is no evidence of record to support appellant allegation. While it is intuitive that genes from a given organism is [*sic.*] optimized for expression in its native cell at certain level to meet the organism' need of said gene product, said gene is not definitely optimized for maximum expression in its native cell.

The Examiner has misconstrued Appellants' statement. In the above-quoted sentence (when read in the context of the entire second paragraph on page 6 of the Brief), Appellants do not allege that a given sequence is naturally optimized for maximum protein expression. Rather, Appellants pointed out that the level of human cell expression of human α-galactosidase, a housekeeping gene that evolved for expression at a native level in human cells, could be increased by optimizing the gene to the extent specifically recited. This is the basis of Appellants' claimed invention.

As discussed above, Seed teaches the optimization of the non-human viral gene, HIV-1 gp120, which is not a human gene. While it is true that HIV-1 gp120 is expressed in HIV-infected human cells, the expression of HIV-1 in humans is a very recent evolutionary event. In fact, a publication by Moore[1] (*American Scientist* 92:540-547, 2004, copy enclosed) states that HIV-1 expression in humans did not occur until the 20th century. Thus, Seed's teaching to optimize a viral gene, i.e., a foreign gene only recently expressed in human cells, and not in normal cells but rather virally infected cells, would have provided no motivation to a skilled practitioner to optimize human α-galactosidase, i.e., a housekeeping gene that has evolved over a very long period of time to be expressed in a broad spectrum of human cells at a level sufficient to provide enough α-galactosidase in the human body to perform its critical function.

---

[1] Appellants submit this reference only in support of previously made arguments that viral sequences (e.g., HIV-1) are less evolved to be expressed in human cells than human sequences.

For the reasons discussed above, Seed does not provide a motivation to one of skill in the art to arrive at the claimed invention. The two genes and, more importantly, the relationship of each to a human cell, are very different.

Kim

The Examiner (Answer at page 11) states that

> Kim is relied upon for the teaching that the expression of a native human gene can be highly optimized by replacing the non- and un-prefered codone [*sic.*] with preferred codones [*sic.*]. In spite of the increased content of CpG dinuclutide [*sic.*] in the synthetic gene relative to the wild type human gene, the synthetic gene is expressed at high levels in human cells.

Appellants respectfully disagree with this characterization.

Kim teaches human erythropoietin (EPO) sequences in which the non-preferred codons are replaced with preferred human codons or preferred yeast codons (*see* Figure 2, page 295). Kim also teaches a chimeric human EPO sequence in which the amino-terminal non-preferred codons are replaced with preferred yeast codons and the remaining non-preferred codons are replaced with preferred human codons. Kim provides a comparison of the expression of these three optimized sequences (*see* Figures 4, 5 and 6). What Kim fails to demonstrate is that the EPO sequence with preferred human codons is expressed at higher levels than the native EPO sequence (containing no preferred human codon replacements). That experiment was simply not done in Kim. The results demonstrate that the EPO sequence with preferred human codons is expressed at a higher level than the EPO sequence with preferred yeast codons. Again, there is no comparison to a native human EPO sequence. Most notably, the results demonstrate that the chimeric EPO sequence with preferred yeast and preferred human codons (i.e., a less optimized sequence) is expressed at even higher levels. Thus, the best results were with the "less" optimized sequence. Appellants submit that the Examiner's statement that "the synthetic gene is expressed at high levels in human cells" does not reflect the teaching of the reference. Kim demonstrates that the expression of

EPO with preferred human codons is higher relative to that optimized with preferred yeast codons.

The Examiner misconstrues these results to mean that the expression of native human EPO can be optimized by replacing non-preferred human codons with preferred human codons. The authors of the Kim publication never stated this conclusion. In fact, Kim provides guidance that optimization with only preferred human codons is not desirable, but rather that the best expression is achieved with a human gene optimized with both preferred yeast and preferred human codons. Thus, Kim does not provide the necessary motivation to one of skill in the art to arrive at the claimed invention.

Seed and Kim, individually or in combination, fail to suggest the nucleic acids of the claims. The other references relied on by the Examiner, Morgan, Bishop and Wada, fail to add anything, and specifically fail to suggest the use of high levels of common codons required and thus fail to remedy the shortcomings of Seed and/or Kim.

Seed and Kim - Teaching Away

The Examiner has reiterated the Office's position that neither Seed nor Kim teach away from the present invention and that therefore, the pending claims are obvious in view of these references in combination with Morgan, Bishop and Wada. While the Examiner has agreed that both Seed and Kim include statements that caution away from the present invention (*see* Answer at page 9, lines 15 to 17), the Examiner concludes that they are of less importance and hold less weight than the experimental results described in those references. Specifically, the Examiner states (Answer at page 10, lines 6 to 11 and 27 to 29):

> Appellants content [*sic.*] that the mere presence of cautions in the reference(s) leads away from substantial increased use of CpG pairs.
>
> * * *
>
> While these cautionary remarks must be considered, it has to be considered and weighed against objective evidence of record.
>
> * * *
>
> In the instant case, both reference [*sic.*] provided the cautionary remark, but they went a head [*sic.*] and substantially increased CpG dinucleotide in

> their synthetic gene relative to the wild type any way [*sic.*], and achieving spectacular results.

The Examiner goes on to state that "[a] cautionary remark in the prior art, which the authors of the prior art acted against, does not rise to the same level of experimental results" (Answer at page 11, lines 9 to 11).

Appellants respectfully disagree that the statements in Seed and Kim, which clearly caution against and lead away from the present invention, are somehow negated or outweighed by Seed's and Kim's experimental data. Appellants submit that the Examiner has given far too little weight to these statements and far too much weight to the experimental results of Seed and Kim, which, as discussed above, the Examiner has misconstrued.

Characterizing Appellants' argument as a simple assertion that the "mere presence of cautions in the reference(s) leads away" from the claimed invention understates Appellants' position and the importance of Seed's and Kim's statements. Appellants believe that these statements are not simple pointers thrown into these two publications as a mere afterthought. Rather, Appellants note that the statements are essentially consistent with each other and submit that they reflect a general principle known in the art at the time the present application was filed, i.e., that the presence of too many CpG dinucleotides in nucleotide sequences results in decreased protein expression. Appellants believe these statements were made in Seed and Kim to guide skilled practitioners in applying that principle to the systems they describe.

Skilled practitioners, knowing that CpG dinucleotides generally inhibit protein expression and being advised of such by Seed and Kim, would have read the experimental results of Seed and Kim as describing only two systems where the principle might not be true. However, neither Seed nor Kim provides any teaching that a human gene can be expressed at higher levels, relative to a native human gene, by replacing the less common codons with common codons without regard to CpG dinucleotides. One of skill in the art, having read Seed's and Kim's experimental data, would have had no reason to expect that replacing the less common codons of a human α-galactosidase sequence with common codons would result in higher α-galactosidase protein expression

levels, given Seed's and Kim's clear statements leading away from doing so and lack of guidance for doing so.

Thus, Appellants respectfully submit that Seed's and Kim's statements are no less important and clearly do not hold less weight than the experimental results described in these references. Appellants maintain their position that Seed and Kim clearly teach away from the present invention.

Finally, Appellants respectfully point out that the Examiner states (Answer at page 11, lines 11 to 13, emphasis added):

> Thus, one of ordinary skill in the art would have considered the cautionary remarks of Seed and Kim in view of their spectacular successes in the laboratory, and carry out the claimed invention any way.

The Examiner appears to be arguing that, in view of Seed's and Kim's experimental results with HIV-1 gp120 and EPO, it would have been "obvious to try" replacing the non-common codons with common codons in a human α-galactosidase nucleic acid sequence, thus supposedly arriving at Appellants' invention. However, as the Examiner is aware, "obvious to try" is not the proper standard in determining whether a claim is obvious under 35 U.S.C. § 103. As such, Appellants respectfully submit that the Examiner, in addition to misconstruing the teachings of the prior art references and dismissing the teaching away of the references, also appears to be using an improper basis for finding the claims obvious over the prior art of record.

For these reasons, and the reasons stated in the Brief In Appeal, Appellants submit that the final rejection should be reversed.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 10 Oct 04

Louis Myers
Reg. No. 35,965

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

The Puzzling Origins of AIDS

Although no one explanation has been universally accepted, four rival theories provide some important lessons

Jim Moore

Shortly after the 1983 discovery of the human immunodeficiency virus (HIV), the pathogen responsible for AIDS, investigators became aware of a strangely similar immune deficiency disease afflicting Asian monkeys (macaques) held in captivity in various U.S. research labs. Soon, virologists identified the culprit: a simian immunodeficiency virus (SIV) that is found naturally in a West African monkey species, the sooty mangabey *(Cercocebus atys)*, but is harmless to that host. This virus, denoted SIVsm, is genetically similar to a weakly contagious form of the AIDS virus that is largely restricted to parts of West Africa, HIV-2, and thus is considered its likely precursor. More recent work has shown that the closest relative of the primary human immunodeficiency virus (HIV-1) is another simian immunodeficiency virus, one carried by chimpanzees (SIVcpz).

After comparing the SIVs in chimpanzees and sooty mangabeys with HIV-1 and HIV-2 strains, investigators concluded that there must have been multiple transmission "events" from simians to humans—at least seven for HIV-2 (some of which are known from only a single person who lives near mangabeys carrying a uniquely similar SIV) and three for HIV-1, the virus now infecting some 40 million people worldwide.

How did SIVcpz and SIVsm cross over into humans and become pathogenic? Given the lack of historical references to AIDS-like disease in Africa prior to the mid-20th century, as well as its absence previously in the New World (which imported some 10 million African slaves during the 16th through 19th centuries), that transfer appears to have happened relatively recently—exactly when is a point of considerable debate. And why did two distinct simian viruses with which humans have apparently coexisted for centuries, or even millennia, suddenly pass into humans multiple times within a few decades?

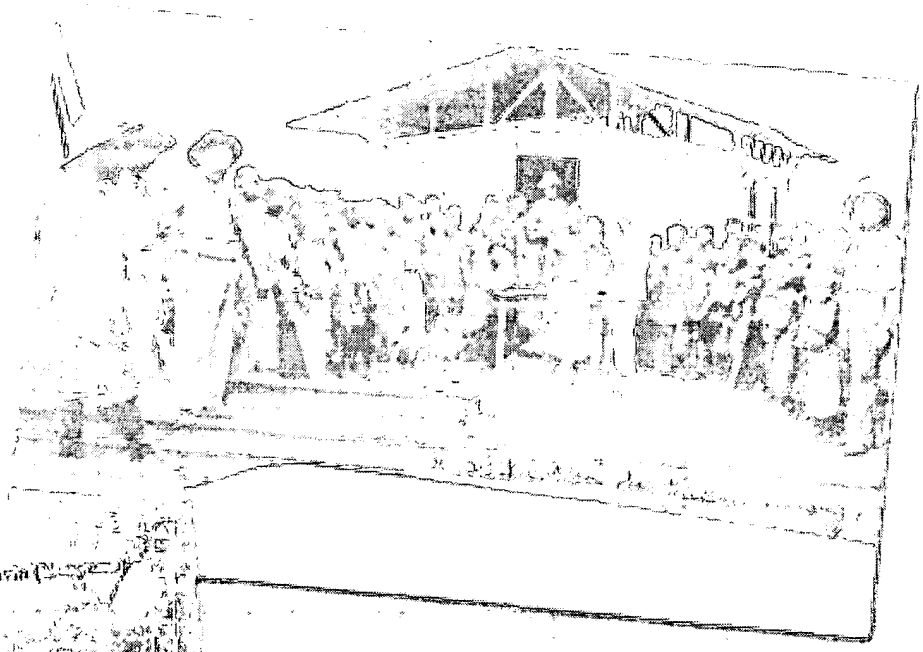
The answers to these questions have been slow in coming, despite the considerable efforts of molecular biologists to understand the nature and evolution of primate immunodeficiency viruses. I am not one of those molecular biologists; rather, I became a player in the field of AIDS-origin research through my interest in chimpanzee socioecology. Although I am partial to a theory I helped to fashion for why AIDS emerged when it did, with time it might become clear that a competing idea better accounts for genesis of the epidemic. Or perhaps the answer will prove to lie with some complex combination of factors that no single explanation presently encompasses. Whatever the case, the solution almost certainly will come from one or more of four competing theories.

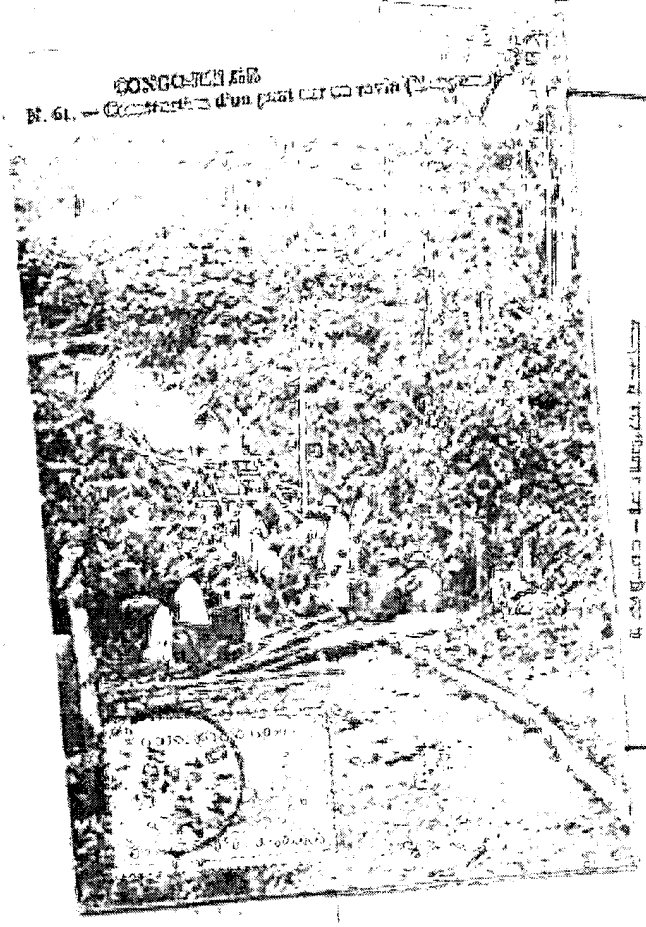
Theory 1: Tainted Polio Vaccine

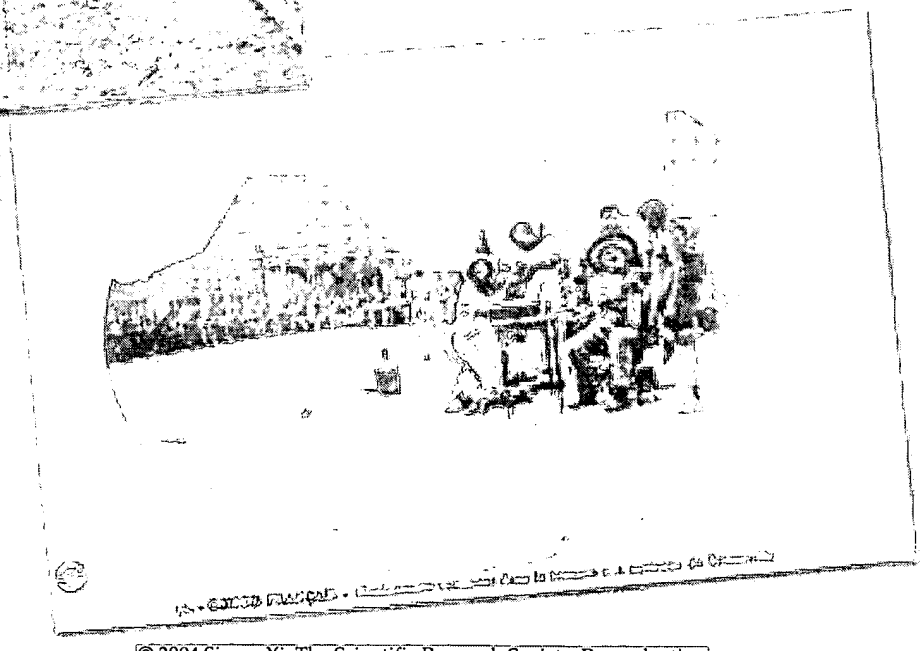
The first theory is the most controversial. In a 1992 article in the magazine *Rolling Stone*, journalist Tom Curtis suggested that HIV could have resulted from the use in Africa of an experimental oral polio vaccine (OPV), one contaminated by a then-unknown SIV carried most probably (Curtis supposed) by African green monkeys. Green-monkey kidney cells were widely used as a substrate to grow viruses for research and vaccine production. And one of the first major trials of an experimental oral polio virus vaccine took place from 1957 to 1960 in what are now the Democratic Republic of the Congo, Burundi and Rwanda, seemingly the "hearth" of the global AIDS epidemic. When interviewed by Curtis, Hilary Koprowski, the polio-vaccine pioneer who mounted that massive campaign, could not recall or find documentary evidence as to whether his group had used kidney cells from green monkeys or Asian macaques (which do not naturally carry an SIV). If culture media contained SIV (a possibility, given that the techniques available during that era were unable

Figure 1. Investigators puzzle over why the AIDS epidemic struck when it did. A simian virus very similar to HIV-1 (the HIV type responsible for the vast majority of AIDS cases) is found in the chimpanzees of Central Africa, suggesting that these animals naturally harbor the progenitor virus. The leading idea is that this virus first passed through cuts to someone hunting or butchering a chimpanzee, but this theory alone cannot explain why the AIDS epidemic did not arise before the 20th century, because hunting chimpanzees for meat has presumably been going on for thousands of years. The author and two of his students suggested that the forced labor and population movements imposed on the natives of Central Africa during the colonial era—and the unsterilized needles used in health campaigns associated with those disruptions—might have created conditions favoring the transfer of the progenitor virus from chimpanzees to humans and its adaptation to become HIV. Vintage postcards show some relevant scenes from the region during the early part of the 20th century. Clockwise from top: vaccination in the Congo Free State (now Democratic Republic of the Congo); Haute-Sangha (a province of what is now Central African Republic), doctor vaccinates natives in the field; French Congo (now Republic of the Congo), missionaries vaccinate in the field in the vicinity of Brazzaville; Belgian Congo (now Democratic Republic of the Congo), construction of a bridge over a ravine.

Jim Moore received his doctorate in biological anthropology from Harvard in 1985, where he studied demography and sociality in primates. Since then he has been on the faculty of the University of California, San Diego, where his research focuses on primate behavioral ecology. Address: Anthropology Department, University of California, San Diego, La Jolla, CA 92093. Internet: jjmoore@ucsd.edu







to guard against unknown viruses that did not cause overt symptoms in their monkey hosts), more than 900,000 people might have received it with their medicine, laying the basis for the current epidemic.

Curtis credited this theory to Blaine Elswood, a Californian AIDS activist. Interestingly, the idea that the administration of a contaminated oral polio vaccine might have been involved in the genesis of AIDS was suggested independently by two others at about the same time. The first to do so was Louis Pascal, who like Elswood is not a scientist. After years of rejections, Pascal, a New Yorker, finally managed in 1991 to get the University of Wollongong in Australia to publish a paper describing his ideas. Not surprisingly, few noticed it. Attorney Walter Kyle also published a broadly similar theory in *The Lancet*, a British medical journal, in 1992. Since then, writer Edward Hooper, author of the controversial 1999 book *The River*, has become the contaminated-vaccine theory's most ardent supporter. Hooper, noting a passing mention by Curtis of a chimpanzee colony run by Koprowski's team, suggested that kidneys from these chimpanzees—not from green monkeys—may have been the original source of the virus.

Multiple localized strains of HIV have now been discovered, and mass vaccination appears unlikely to account for all of them. But the early distribution of the major pandemic strain, HIV-1 group M (for "main"), seems to fit reasonably well with the location of Koprowski's campaigns, and the OPV theory now is applied primarily to this strain.

Contamination of OPV is the only one of the four current theories that is readily falsifiable: Finding the HIV-1 group M virus in a tissue sample that predated the suspect vaccine would eliminate this possibility. So far that has not happened. Still, many investigators give the theory little weight for other reasons, which has led to the widespread belief that the theory has been definitively disproved. In 2001, for example, *Science* magazine published a piece titled "Disputed AIDS Theory Dies its Final Death," and *Nature* ran one under the heading "Polio Vaccines Exonerated." Earlier this year *Nature* also published "Origin of AIDS: Contaminated Polio Vaccine Theory Refuted"—a surprising title given that this theory ostensibly died three years ago.

The recent findings of various molecular biologists have indeed failed to provide support for the OPV theory. For example, in 2000 a few existing samples of the vaccine from Koprowski's home institution (the Wistar Institute in Philadelphia) were tested and found negative for both chimpanzee DNA and SIV. However, this result did not rule out the possibility, previously suggested by Hooper, that local amplification of the live-virus vaccine in Africa (to create more doses) could have introduced the SIV. The key issue is thus whether chimpanzee kidneys were used as a culture medium at any stage of Koprowski's vaccine program. There is eyewitness testimony on both sides of this question, and failure to find SIVcpz in a handful of samples of the live vaccine strain of the type used in Africa does not prove the virus was absent in (putative) locally produced batches.

A second reason to question the OPV theory also came to light in 2000, with a report in *Science* by Bette T. Korber (of Los Alamos National Laboratory) and colleagues. They used molecular differences among HIV-1 group M subtypes to estimate the date of their last common ancestor. The conclusion: 1931 (with 95 percent confidence limits giving the range 1915 to 1941), preceding OPV administration by decades. However, the calculation of such common-ancestor dates can be thrown off by genetic recombination among subtypes ("viral sex"), which can make such dates come out too early, and there is increasing evidence that such recombination may be common with HIV. So maybe this date is not right. On the other hand, independent analyses using different methods have supported the date, and an analogous study of HIV-2 came up with an origin for the main group between 1940 and 1945.

Another objection to the OPV theory concerns the subspecies of chimpanzee kept near Kisangani (formerly Stanleyville) at a facility called Camp Lindi, which Koprowski and colleagues maintain was used for safety-testing their vaccine, but which Hooper suspects was the source of chimpanzee tissues used to produce vaccine locally. The SIVcpz strain that is most similar to HIV-1 has so far only been identified in a subspecies of chimpanzee native to west-central Africa, *Pan troglodytes troglodytes*. A second, less similar strain has been identified only in *Pan troglodytes schweinfurthii*, the subspecies found in east-central Africa—where Camp Lindi was located. The nearest known populations of *P. t. troglodytes* are more than 500 kilometers from Koprowski's chimp colony. So, this argument goes, the locally obtained captive chimps would not

Figure 2. One controversial theory posits that the transfer of the chimpanzee immunodeficiency virus to human beings took place between 1957 and 1960 in the course of an oral polio-vaccination campaign carried out by Ghislain Courtois, Hilary Koprowski and their colleagues in what are now the Democratic Republic of the Congo, Burundi and Rwanda. This sign from the chimpanzee colony maintained in connection with that campaign reads, "Polio mission of Courtois-Koprowski, experimentation center, entrance forbidden." (Photograph by Gilbert Rollais, courtesy of Edward Hooper.)

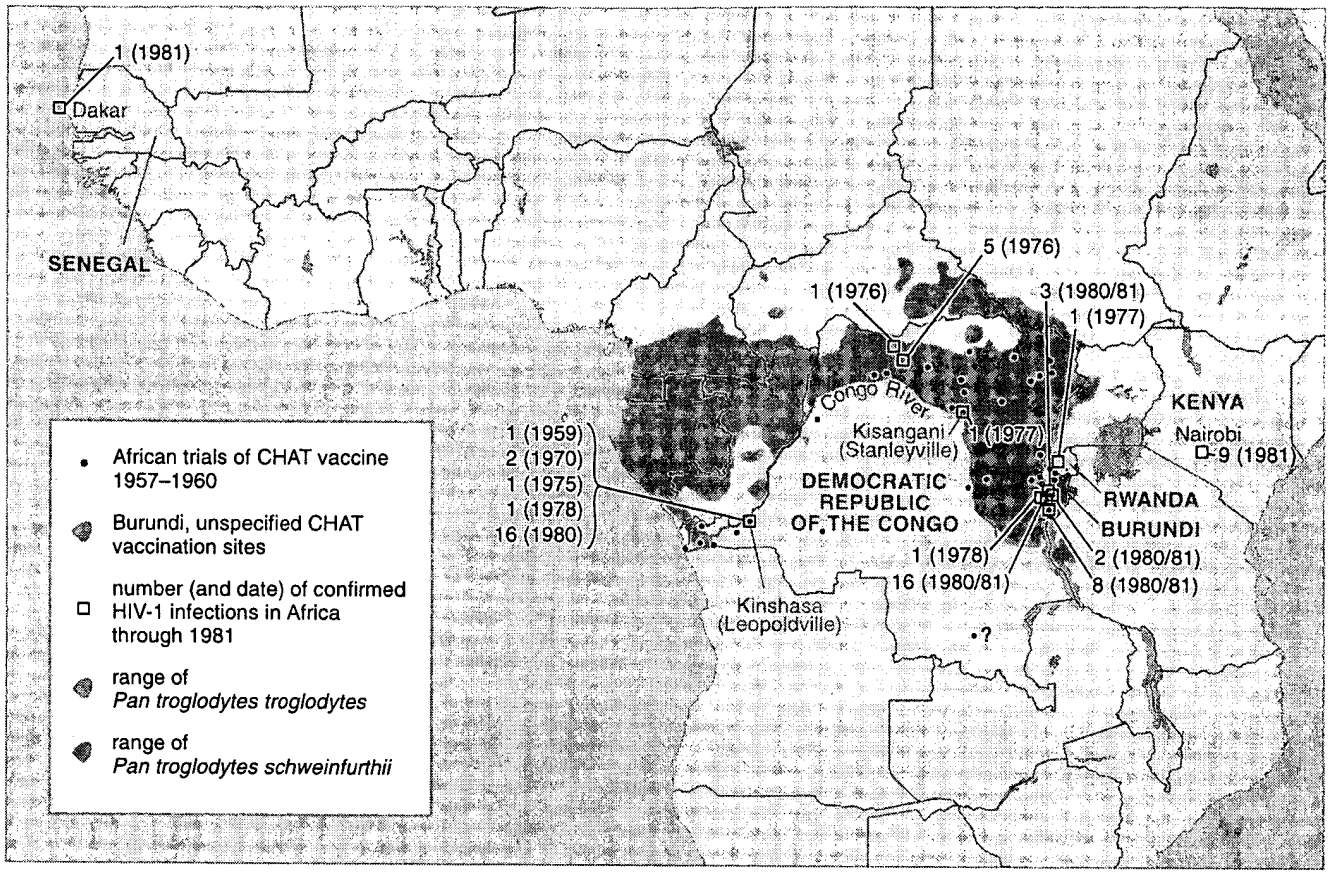


Figure 3. "CHAT" oral polio vaccine was fed to approximately one million people at various sites *(red dots* and *pink zone)* between 1957 and 1960. The degree of correspondence between these locales and early evidence of HIV-1 infection in Africa through 1981 *(squares)* is striking. The evidence comes either from patients who showed symptoms of AIDS and who later proved to be infected with HIV-1, or from HIV-positive blood samples taken at the time. (Note that two confirmed AIDS cases are not shown: a patient who acquired the virus somewhere in Tanzania before 1981, and one who acquired a form of the virus that is genetically distinct from the main form in either Cameroon or Kenya before 1967.) A comparison of CHAT sites and early AIDS cases that were never serologically tested *(not shown)* gives a similarly high degree of correspondence. Critics of the theory that this vaccination program ignited the epidemic note that the correlation between vaccination sites and early evidence of AIDS may just reflect the distribution of population centers and of medical facilities. They also point out that the SIVcpz carried by *Pan troglodytes schweinfurthii (green)*—the subspecies of chimpanzee found near Stanleyville (Kisangani), where those involved with the CHAT campaign maintained a colony of chimpanzees—is less closely related to HIV-1 than is the SIVcpz carried by *Pan troglodytes troglodytes*, which lives to the west *(purple)*. The first criticism requires a careful statistical analysis to evaluate. The second ignores the fact that some chimpanzees might have been obtained for the colony from hunters working lower on the Congo River, which supported considerable steamer traffic at the time. (Data on CHAT sites and early HIV occurrences are from Hooper 2000. Subspecies ranges derived from Worobey *et al.* 2004.)

have been carrying the SIVcpz strain thought to have given rise to HIV-1.

One difficulty with this argument is that distance is not always measured in kilometers, particularly in Central Africa: Kisangani lies at the upstream end of the navigable portion of the Congo River, which borders the range of *P. t. troglodytes* for hundreds of kilometers, and river trade has been substantial since the colonial scramble for Africa in the late 19th century. If it became known that Americans were paying good money for young apes in Kisangani, it would be almost surprising if some hunters had not made the trip upriver. Another problem is the difficulty of proving the absence of something based on only a few samples, which requires some significant assumptions about the epidemiology of SIVcpz in the wild.

In short, although the majority of the biological evidence published in the last few years suggests that the OPV hypothesis is wrong, headlines reporting the death of this theory remain premature.

Theory 2: Cut Hunter

The main competing theory posits that SIV is occasionally transmitted to hunters via blood-to-blood contact with an infected primate. According to this view, the virus is usually cleared in its human host, but at least several times during the 20th century it survived and became established as HIV. It is not hard to imagine hunters suffering cuts or being injured by a wounded mangabey or chimpanzee, and some form of natural transfer between species presumably accounts for the widespread distribution of SIVs in African primates. Hence, one has the "cut hunter" or "natural transfer" theory, which is probably the most accepted idea today. According to that view, the timing of the widespread emergences of HIV-1 and HIV-2 in the middle part of the 20th century is attributed to urbanization and regional commerce, which create conditions ideal for spreading a sexually transmitted disease.

Unlike the case with OPV, there is no easy way to disprove this theory—even a smoking gun linking oral polio vaccines to HIV-1 group M would leave multiple other HIV strains unaccounted for, and "modernization" is a diffuse enough explanation to cover

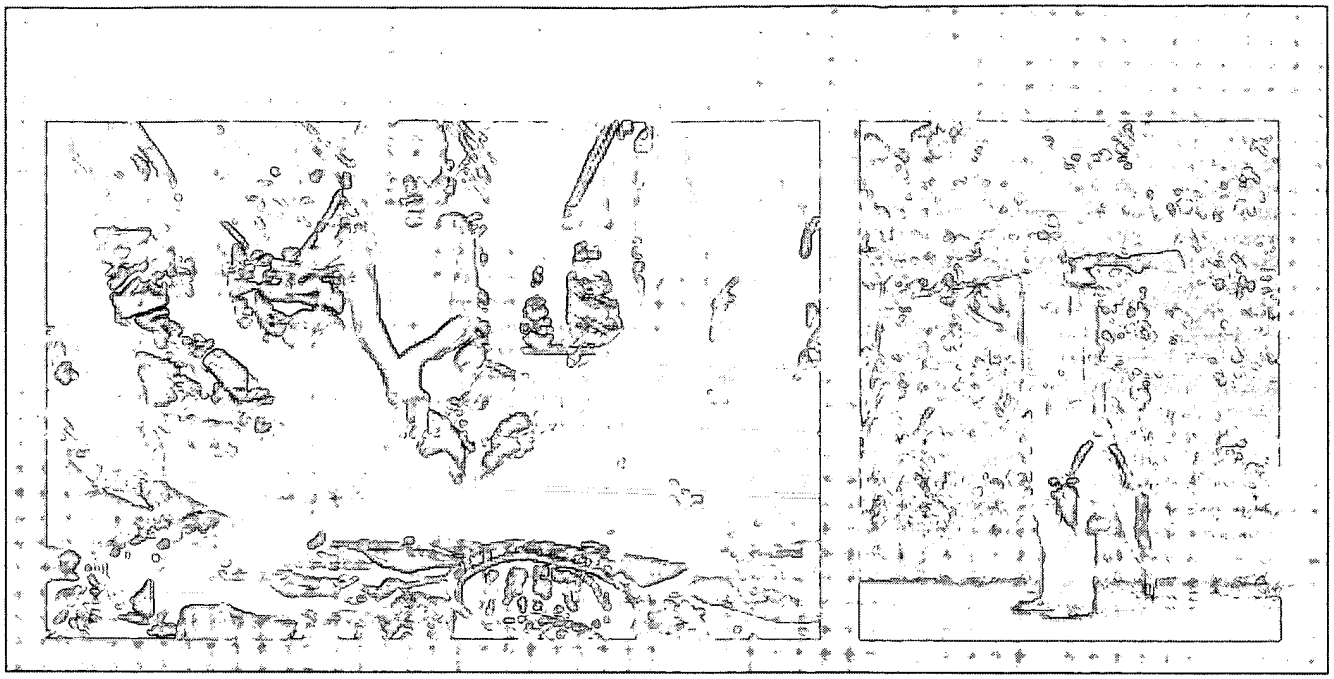


Figure 4. SIV may have crossed the species barrier to humans in the course of someone killing a chimpanzee or monkey for meat. At left, Efe Pygmy hunters of the Ituri Forest, Democratic Republic of the Congo, butcher a mangabey killed with bow and arrow. At right, a hunter from Sierra Leone uses a more modern weapon (a shotgun) to kill mangabeys. (Photograph at left by Heidi Verhoef, courtesy of the Bushmeat Crisis Task Force, www.bushmeat.org. Photograph at right courtesy of Glyn Davies, Zoological Society of London.)

any of them. Nor is the cut-hunter theory particularly limited in time. After all, many Africans began moving to colonial capitals and ports in the 19th century. A hypothesis that does not account for the timing of the AIDS epidemic and that is not falsifiable is of limited use. Still, the thinness of the theory does not make it wrong

Theory 3: Contaminated Needles

The next proposal, a refinement of the cut-hunter theory, comes from Preston A. Marx, a virologist who holds positions at Tulane University and at the Aaron Diamond AIDS Research Center. In 1995 he noted (to Hooper) that a big change in medical practice took place in the 1950s with the worldwide introduction of disposable plastic syringes, making guaranteed sterile use possible and dropping the cost of syringe production by almost two orders of magnitude. The result was that the medical use of injections went up astronomically. Because doses can be measured and there is no possibility of patients losing or selling the medicine, injections became a popular way for doctors in the developing world to administer medicines, including vitamins, analgesics and other common drugs.

The problem is that trivial costs are still large to someone living outside the cash economy, and plastic syringes *cannot* be sterilized by boiling: they melt. According to this scenario, the widespread availability of disposable syringes increased the acceptance of injections to treat a variety of diseases, but the syringes were not so available (or cheap) as to permit users actually to dispose of them. The result was that unsterilized syringes were used again and again, spreading viruses, including those that eventually became HIV.

Marx suggests that people's immune systems would normally be able to overcome an SIV they acquired, say while butchering a monkey, within a week or two of infection. He further posits that the transition from SIV to HIV demands a series of mutations, with the probability of all the required mutations occurring being a function of viral population size. Thus, Marx contends, some way must be found to permit the SIV to remain at high levels in people for long enough that such spontaneous mutations might take place. He suggests that the required mechanism is "serial passaging" of virus through unsterile needles. That is, a cut hunter might get an injection while he is still harboring large numbers of viral particles in his bloodstream; that same needle would then be used to infect another person, who might soon receive a second injection, and so forth. High viral population levels can thus be maintained in a series of different people getting shots. With each transfer via contaminated needle, the virus finds itself in a fresh host, with an opportunity to proliferate before the infected person can mount an immune response. Chance mutations can thus accumulate, and eventually the SIV adapts, becoming HIV.

Theory 4: *Heart of Darkness*

Together with two undergraduate students, I am responsible for another variant to the cut-hunter theory, so perhaps I should explain how I became engaged in this field of inquiry. In late 1998 I became involved in an

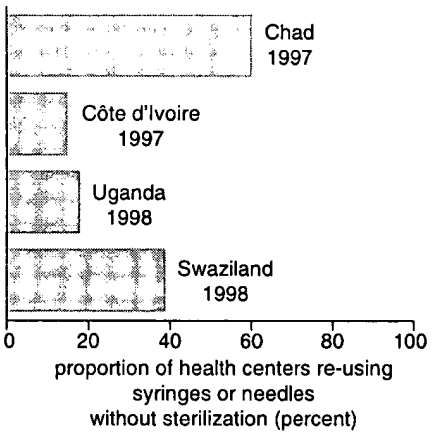


Figure 5. Because the cut-hunter theory alone fails to explain the timing of the AIDS epidemic, investigators have looked for other factors that might hold the key. One is the widespread distribution of disposable syringes, which began in the 1950s. Although inexpensive, these devices proved too precious to be thrown out in many poor parts of the world. And unlike the metal or glass units that they replaced, plastic syringes cannot be sterilized by boiling them (they melt). The result is that unsterilized syringes were often reused, spreading disease. Even now, such problems are common in the developing world, as can be seen in the proportion of health centers in selected African countries where syringes or needles are reused without sterilization. (Data from Dicko *et al.* 2000.)

e-mail discussion about the conservation implications of the identification of central African chimpanzees as the source of HIV-1, a result that Beatrice H. Hahn of the University of Alabama at Birmingham and her colleagues had just published. At about the same time, a colleague urged me to read *King Leopold's Ghost*, Adam Hochschild's history of the Belgian Congo, and I was independently contacted by two students, Amit Chitnis and Diana Rawls, who were interested in doing something involving the intersection of biological anthropology and medicine. Then came the catalyst: an article in *Discover* magazine that mentioned the idea that the origin of AIDS might have had something to do with the chaos that followed colonial withdrawal from central Africa. The notion was that the colonial authorities had kept things under control, but when they left, "there was a free-for-all" that provided the conditions for the establishment of a new disease.

King Leopold's Ghost had more impact on me than any other book I have read. I had vaguely heard that Belgian rule was harsh, but I had not realized that more Africans probably died as a result of colonial practices in French Equatorial Africa and neighboring Belgian Congo between 1880 and the onset of World War II than had been taken from Africa as slaves during the preceding 400 years. "Probably," because no record was kept of the dead. The first censuses, taken in the 1920s, estimated that the population of the two colonies was then about 15 million. Census-takers recorded that wherever they asked, local people (colonial and native) reported that about twice as many had lived there two or three decades before, indicating that some 15 million had died. Losing 50 percent of the population exceeds even the 35-percent fatality rate of the Black Death in Europe.

It seems Joseph Conrad's *Heart of Darkness* was as much fact as fiction, and the horror described in that famous novel reflected official policies in the Congo as much as individual insanity. What appeared to many as colonial "control" of the region in the late 19th and early 20th centuries brought chaos to the lives of the Africans who lived and died under it. Chitnis, Rawls and I set out to see what disease-promoting factors might have existed prior to the withdrawal of colonial powers around 1960.

Candidates were not difficult to find, at least during the years prior to World War I. Forced labor camps of thousands had poor sanitation, poor diet and exhausting labor demands. It is hard to imagine better conditions for the establishment of an immune-deficiency disease. Where imagination fails, let history serve. To care for the health of the laborers, well-meaning but undersupplied doctors routinely inoculated workers against smallpox and dysentery, and they treated sleeping sickness with serial injections. The problem is, the multiple injections given to arriving gangs of tens or hundreds were administered with only a handful of syringes. The importance of sterile technique was known but not regularly practiced: Transfer of pathogens would have been inevitable. And to appease the laborers, in some of the camps sex workers were officially encouraged.

And that was just the situation in the camps. Major efforts were made to eradicate smallpox and sleeping sickness

Stock Montage

Figure 6. Another modification of the cut-hunter theory suggests that the widespread brutalization of natives of the Congo basin during the colonial era promoted both the adaptation of SIVcpz to humans (its transformation to HIV-1) and the initial spread of the virus. In particular, people living in this region suffered enormously, many being forced to extract ivory and rubber from the jungle. King Leopold II of Belgium came under intense international scrutiny as a result of his harsh treatment of those living in the Congo Free State. This drawing, which appeared in the magazine *Punch* in 1906, shows a native man ensnared by a serpent with the head of King Leopold.

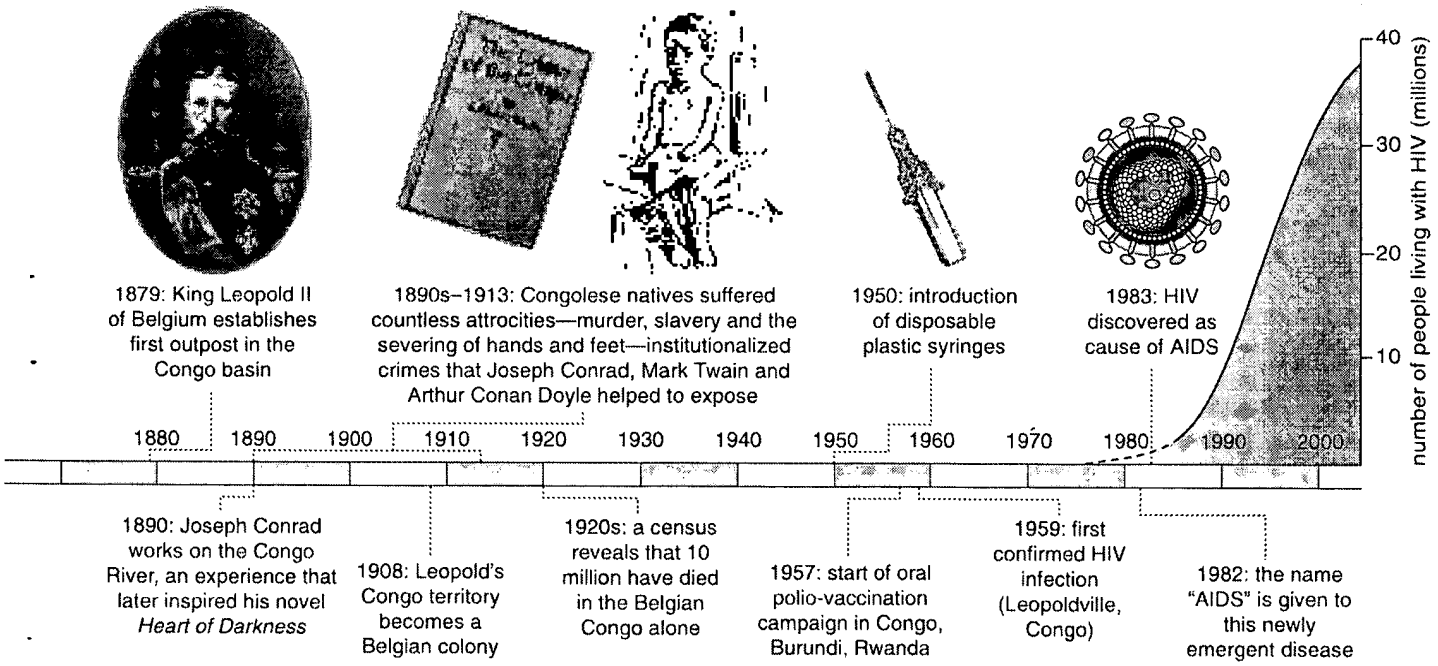


Figure 7. Different theories point to different events as crucial to the genesis of the AIDS epidemic. The colonial-disruptions theory emphasizes goings-on in the early part of the 20th century, whereas the contaminated-needle theory places the spark after 1950. The controversial theory attributing the epidemic to an experimental polio-vaccination campaign carried out between 1957 and 1960 falls closest in time to the first confirmed HIV-positive blood sample, taken in 1959 from someone living in Leopoldville (Kinshasa). The number of people infected with HIV has since risen to almost 40 million. (Data on rise in HIV infections from UNAIDS.)

Jeffrey B. Joy

Figure 8. Until his death in 2000, William D. Hamilton, a renowned evolutionary biologist at the University of Oxford, was the most prominent scientist expressing support of the OPV theory. He died as an indirect result of malaria acquired in the Congo, where he and two coworkers were collecting material to probe the detailed nature of simian immunodeficiency viruses in a region where chimpanzees were obtained in conjunction with the polio-vaccination campaigns of Koprowski and his colleagues. This photograph shows Hamilton in the field during his final expedition.

elsewhere in the region (these diseases cut into productivity). The shortage of syringes was acute. One 1916 sleeping-sickness control expedition treated 89,000 people in Ubangi Shari (now Central African Republic) using just six syringes. And before the introduction of dried smallpox vaccine in about 1914, the only way to transport vaccine to the interior was by serially inoculating people, traveling during the eight-day interval required for the new carrier to develop pustules from which the next inoculation could be derived. There are records of at least 14,000 people receiving vaccine in this way. The method had been abandoned in Europe some 20 years before, because syphilis was all-too-often transmitted accidentally in the process.

Such circumstances easily could have promoted the evolution of HIV from SIVcpz. Imagine, for example, the following scenario:

A fisherman flees his small village to escape a colonial patrol demanding its rubber quota; as he runs, he grabs one of the unfamiliar shotguns recently arrived in the area. While hiding for several days, he shoots a chimpanzee and, unfamiliar with the process of butchering it, is infected with SIVcpz. On return to the village he finds his family massacred and the village disbanded. He wanders for miles, dodging patrols, until arriving at a distant village. The next day he is seized by a railroad press gang and marched for days to the labor site, where he (along with several hundred others) receives several injections for reasons he does not understand. During his months working on the railroad, he has little to eat and is continually stressed, susceptible to any infection. He finds some solace in one of the camp prostitutes (themselves imported by those in charge), but eventually dies of an undiagnosed wasting—the fate of hundreds in that camp alone. Disease, starvation, abuse—no record is kept, none of the authorities knows, and those few doctors who care are overwhelmed.

We wrote up a short article laying out reasons to at least examine colonial-era practices seriously in regard to how they may have contributed to the origin and spread of HIV. It probably would have been ignored but for another coincidence: Our paper appeared in the journal *AIDS Research and Human Retroviruses* almost simultaneously with the report of Korber and her colleagues in *Science* placing the beginnings of HIV-1 Group M in the early decades of the 20th century. If this dating is correct, the colonial-policy theory offers an explanation. Note, however, that a version of the basic cut-hunter theory that does not rely

on urbanization (or sets a much lower threshold for the critical level of city life) could also explain the genesis and initial spread of HIV during this period.

Neither of these scenarios neatly accounts for the decades between the postulated origin of HIV in the early part of the 20th century and the widespread emergence of AIDS in Africa, which did not take place until the early 1980s. But maybe that long delay is only an artifact of our perceptions: Starting with a single case and assuming a doubling in frequency every few years, one would need decades to pass for the prevalence to build appreciably; would colonial doctors have noticed an initially rare immune disease? Nor do these theories readily explain details of the spatial pattern in the early cases of HIV infection and AIDS, which indeed show a suggestive overlap with the sites of oral polio vaccination. But is that correspondence just a function of the distribution of population and doctors? As with all of the current ideas, one can suggest various explanations to account for intriguing observations or troubling discrepancies. For the moment, the fit between theory and observation remains loose enough that no one view has proved absolutely compelling.

Battling Theories

Arguments over rival theories of the origin of AIDS have raged viciously at times—far beyond the norms of most scientific debates. Indeed, both sides in the OPV controversy have in the recent scientific literature gone so far as to accuse their opponents of lying and manipulating evidence. I only became aware of the explosive nature of the debate after my students and I unwittingly wandered into this minefield.

Some of the participants in this controversy appear unwilling even to entertain the possibility of being wrong. Given the precarious status of each of the current theories, it seems more reasonable to try to keep an open mind until better evidence emerges and, in the meantime, to consider the literature on each of these origin stories as representing a highly refined simulation scenario. Insofar as there is any material benefit to come from understanding the origin of HIV in terms of cautionary tales, each model can and should be considered plausible—and worrisome. After all, unsterile needles *do* transmit diseases, contaminated polio vaccine *did* spread a simian virus (one called SV40) to millions of people, doctors *do* sometimes conduct risky research, colonial policies *did* have major health consequences, and contact with wild animals *can* introduce pathogens into humans.

An obvious general lesson can be drawn from all four theories: For some very puzzling reason, the origin of HIV was not fundamentally natural, given that humans apparently failed to acquire an immunodeficiency virus from simians during thousands of years of exposure. Instead, the emergence of HIV involved social change in one form or another: the abuses carried out at the hand of an invading foreign power; abrupt urbanization overwhelming the ability of medical and political authorities to manage the process; the undersupervised transfer of medical technology and half-measures in development programs; doctors taking liberties in distributing medicines without adequate precautions. It is worth noting that three of the four theories postulate an origin for AIDS that involves the inadvertent results of medical efforts, with what were then state-of-the-art health programs and technologies carrying with them unforeseen dangers.

Whether understanding the origin of HIV and AIDS is useful for evaluating risks associated with present-day concerns (say, the consumption of wildlife that might be the natural reservoir for emerging diseases like SARS, or evaluating the likelihood that the transplantation of animal organs into people will unleash a dangerous new virus) is a matter of opinion. My own view is that a firmer grasp of what happened in the past—and what might easily have happened had circumstances been slightly different—helps society to understand these dangers and to minimize the risk of sparking the next global scourge.

Bibliography

Apetrei, C., D. L. Robertson and P. A. Marx. 2004. The history of SIVs and AIDS: Epidemiology, phylogeny and biology of isolates from naturally SIV infected non-human primates (NHP) in Africa. *Frontiers in Bioscience* 9:225–254.

Chitnis, A., D. Rawls and J. Moore. 2000. Origin of HIV-1 in colonial French Equatorial Africa? *AIDS Research and Human Retroviruses* 16:5–8.

Cohen, J. 2001. Disputed AIDS theory dies its final death. *Science* 292:615.

Curtis, T. 1992. The Origin of AIDS. *Rolling Stone* issue 626 (19 March):54–59+.

Dicko, M., A.-Q. O. Oni, S. Ganivet, S. Kone, L. Pierre and B. Jacquet. 2000. Safety of immunization injections in Africa: Not simply a problem of logistics. *Bulletin of the World Health Organization* 78:163–169.

Hochschild, A. 1998. *King Leopold's Ghost: A Story of Greed, Terror, and Heroism in Colonial Africa*. New York, Boston: Houghton Mifflin.

Hooper, E. 2000. *The River: A Journey to the Source of HIV and AIDS*. Boston: Back Bay Books.

Hooper, E. 2003. Dephlogistication, imperial display, apes, angels, and the return of Monsieur Émile Zola: New developments in the origins of AIDS controversy, including some observations about ways in which the scientific establishment may seek to limit open debate and flow of information on "difficult" issues. *Atti dei Convegni Lincei* 187:27–230.

Korber, B., M. Muldoon, J. Theiler, F. Gao, R. Gupta, A. Lapedes, B. H. Hahn, S. Wolinsky and T. Bhattacharya. 2000. Timing the ancestor of the HIV-1 pandemic strains. *Science* 288:1789–1796.

Kyle, W. S. 1992. Simian retroviruses, polio-vaccine, and origin of AIDS. *The Lancet* 339:600–601.

Lemey, P., O. G. Pybus, B. Wang, N. K. Saksena, M. Salemi and A.-M. Vandamme. 2003. Tracing the origin and history of the HIV-2 epidemic. *Proceedings of the National Academy of Sciences of the U.S.A.* 100:6588–6592.

Peeters, M., V. Courgnaud, B. Abela, P. Auzel, X. Pourrut, F. Bibollet-Ruche, S. Loul, F. Liegeois, C. Butel, D. Koulagna, E. Mpoudi-Ngole, G. M. Shaw, B. H. Hahn and E. Delaporte. 2002. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. *Emerging Infectious Diseases* 8:451–457.

Peterson, D. 2003. *Eating Apes*. Berkeley: University of California Press.

Reeler, A. V. 1990. Injections: A fatal attraction? *Social Science & Medicine* 31:1119–1125.

Salemi, M., K. Strimmer, W. W. Hall, M. Duffy, E. Delaporte, S. Mboup, M. Peeters and A.-M. Vandamme. 2001. Dating the common ancestor of SIVcpz and HIV-1 group M and the origin of HIV-1 subtypes using a new method to uncover clock-like molecular evolution. *The FASEB Journal*. 15:276–278.

Weiss, R. A. 2001. Polio vaccines exonerated. *Nature* 410:1035–1036.

Worobey, M., M. L. Santiago, B. F. Keele, J.-B. N. Ndjango, J. B. Joy, B. L. Labama, B. D. Dhed'a, A. Rambaut, P. M. Sharp, G. M. Shaw, B. H. Hahn. 2004. Contaminated polio vaccine theory refuted. *Nature* 428:820.

For relevant Web links, consult this issue of *American Scientist Online:*

http://www.americanscientist.org/IssueTOC/issue/661